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SHORT NOTES

Isolation and Characterization of Nine Microsatellite Markers for Red-backed Ratsnake, *Elaphe rufodorsata*

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Abstract The red-backed ratsnake (*Elaphe rufodorsata*) is widely distributed in East Asia, especially China. This species is a common snake in plain river network region. In the past several decades, *E. rufodorsata* has dramatically declined due to the effect of human activities and over hunting for traditional Chinese medicine. We developed nine species-specific microsatellite loci in 190 individuals collected from Huzhou, Zhejiang province in China. These markers revealed a high degree of genetic diversity (13–41 alleles per locus) and heterozygosity (H_O ranged from 0.266 to 0.941, and H_E ranged from 0.851 to 0.937). No locus exhibited significant deviations from Hardy–Weinberg equilibrium. There was no evidence of linkage disequilibrium among pairs of loci. These microsatellite markers were described in our study will be valuable tools for the long term management and population-level studies (e.g. the population structure, genetic diversity and variation, individual paternity and evolutionary history) of the species.

Keywords Colubridae, *Elaphe rufodorsata*, Microsatellite markers, Polymorphism, PCR primers

The red-backed ratsnake (*Elaphe rufodorsata*) is a kind of small-sized, viviparous, nontoxic snake can be found in semiaquatic areas, widely distributes in most parts of China, eastern Russia and Korean Peninsula (Zhao 1998). This snake has been declined because of its medical value for traditional Chinese medicine and habitat destruction. As a result, this snake is listed as the state's ‘‘three are’’ (state protection beneficial or of important economic, scientific value of the list of terrestrial wildlife) animal in China by State Forestry Administration of the People's Republic of China. To better address conservation plan and comprehend population genetic information of this species, we developed nine species-specific microsatellite loci that can be used to study the population genetics of *E. rufodorsata*.

In this study, the tissues were took from the tail tip of three unrelated snakes and preserved in 95 % ethanol before brought to Nanjing Normal University from Huzhou, Zhejiang province. Total genomic DNA from three unrelated individuals was extracted with dBIOZOL Genomic DNA Extraction Reagent (Bioer Technology) and dissolved in TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). We used the total genetic DNA to construct the library enriched for AC/AG/GATA/TATC motif microsatellite sequences according to previous study (Hua *et al.*, 2012). The genomic DNA was digested with *Mse* I restriction enzyme and ligated to *Mse* I AFLP adaptor. After diluted the digestion-ligation mixture with ten times water, we amplified it with AFLP adaptor-specific primers *Mse* I-N (5'-GAT GAG TCC TGA GTA AN-3') for 26 cycles and hybridized the DNA product with $(AC)_{10}$, $(AG)_{10}$, $(GATA)_6$, and $(TATC)_{10}$ probes, separately. The microsatellite-enriched DNA fragments were obtained following the protocol (biotin capture method). These DNA fragments were amplified with *Mse* I-N primers, then ligated the PCR fragments into PMD19-T vector (Promega), and transformed into *Escherichia coli* dH5α competent cell (TAKARA Biotechnology). The transformed cells were plated on Luria-Bertani (LB) agar plate containing X-Gal and IPTG for blue-white selection at 37 °C for 16h.

White colonies were transferred into the liquid medium

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containing ampicillin and kept oscillation culture for 6–8 h at 37 °C. The recombinants were screened by PCR in according with Hua *et al.* (2012). We separated PCR products using an ABI PRISM 3500 XL genetic analyzer with a GS350 size standard, and analyzed genotypes by using GENEMARKER V1.85. We tested 190 individuals to analyze microsatellite data using GENETIX V4.0.

These nine sequences containing microsatellite locus were deposited in GenBank (KM597056–KM597064). Polymorphic information of nine microsatellite loci was presented in Table 1. All the nine loci were polymorphic exhibiting 13–41 (mean of 21.667) alleles per locus (Table 1). H_{O} (observed heterozygosities) ranged from 0.266 to 0.941 (mean of 0.658), and H_{E} (expected heterozygosities) ranged from 0.851 to 0.937 (mean of 0.896). The *PIC* (polymorph information content) value ranged from 0.833 to 0.961 (mean of 0.890). MICRO-CHECKER detected no null alleles. No locus exhibited significant deviations from Hardy–Weinberg equilibrium after sequential Bonferroni correction (Rice, 1989). There was no evidence of linkage disequilibrium among pairs of loci in the samples. These microsatellite loci were described in our study would be used for assessing the genetic diversity, population structure and individual paternity. In addition, these microsatellite loci may be helpful to set up effective conservation measures for *E.rufodorsata*.

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References

Hua L., Mao L. X., Chen C., Gao J. F., Lin L. H. 2012. Isolation and characterization of microsatellite loci in the Reevese's butterfly lizard *Leiolepis reevesii* (Agamidae). Conserv Genet Resour, 4: 791–794

Rice W. R. 1989. Analyzing tables of statistical tests. Evolution, 43: 223–225

Wang R. F., Wu Y. Q., Liu Z., Qu Y. F. 2014. Isolation and characterization of microsatellite loci in the Ussuri mamushi (*Gloydius ussuriensis*). Conserv Genet Resour, 6: 783–784

Wu Y. Q., Wang R. F., Lin L. H., Qu Y. F. 2014. Microsatellite primer development for the king ratsnakes *Elaphe carinata* (Colubridae). Conserv Genet Resour, 6: 211–212

Zhao E. M. 1998. Crotalinae. In Zhao EM, Huang MH, Zong Y (Eds.), Fauna Sinica, Reptilia, Vol. 3 (Squamata: Serpentes). Beijing: Science Press

Table 1 Characteristics of nine *Elaphe rufodorsata* microsatellites DNA loci: locus designation, primer sequences, repeat motif, allele sizes, annealing temperature (*T*a), number of alleles (*N*a), observed (H_{O}) and expected (H_{E}) heterozygosities, polymorph information content (*PIC*) and GenBank Accession No.

Locus	Primer sequence(5'-3')	Repeat motifs	Size range	*T*a (°C)	*N*a	H_{O}	H_{E}	*PIC*	GenBank Accession No.
HD70	F:AGTAACGGAGCAAACACAATG R:ATCCAAAGTGAGTGAGTGAGAG	$(CT)_{30}$	91–145	58	20	0.524	0.924	0.961	KM597056
HD248	F:CCAATGCTATGAGTCTGCTGAG R:TTCATTCCGTAGGGCAAAAGC	$(CA)_{24.5}$	197–233	58	15	0.941	0.895	0.883	KM597057
HD418	F:TGAATTGGTAGAGTTTGGTGTG R:GCAAGTCCCAGAGCAAGG	$(GT)_{23}$	221–255	58	17	0.798	0.884	0.872	KM597058
HD589	F:TTCCTGGTGACTGACATTGTG R:TCTTCTTCTGCCTGGACCTC	$(ATCT)_{15.5}$	129–219	58	14	0.665	0.865	0.849	KM597059
HD134	F:ACCACCAGTCATCTCTCTTTC R:TATGCTGGAGATTCAAGTTGTC	$(ATCT)_{15}$	199–317	58	20	0.862	0.882	0.87	KM597060
HD99	F:CATTCCAGATCACCGATCACTC R:GCTTGATGTCCTTACCTGCTC	$(CA)_{25.5}$	159–199	58	13	0.803	0.851	0.833	KM597061
HD452	F:CTGGATGTGGTTCTCAGTGG R:AAAGAAATTGTAGCGTGTAGGC	$(ATAG)_{19.3}$	235–347	58	33	0.505	0.935	0.929	KM597062
HD847	F:CCATCTAGCAGCAGAGTTCAC R:GAGTGGAGTGGAGTGGAGTG	$(ATCT)_{18.3}$	257–331	58	22	0.56	0.892	0.88	KM597063
HD172	F:GGCTTGGCTTGACATGCTAG R:TCTGGAATCCTCCGTATCTCTG	$(GATA)_{25.3}$	243–545	58	41	0.266	0.937	0.932	KM597064

The 9 microsatellite sequences are as following:
>KM597056
AGTAACGGAGCAAACACAATGCTGCTGCAAATTCATCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCACTCACTCACTTTGGAT
>KM597057
TTCCTGGTGACTGACATTGTGTAGGTGTGTATATCTCTATCTCTATCATCTCTGTCTATCTATCTATCTATCTAT
CTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCGGAAAAGAAGGAAGGCAAATGAGGTCCAGGCA
GAAGAAGA
>KM597058
CATTCCAGATCACCGATCACTCTGCCATTTTTTTTACTGCTACTTTTGTTCTCTGTACACACACACACACACA
CACACACACACACACACACACACACAAACACACAAAACCAAAAACATTATGGAGAGAAAGATGGAAGAG
CCACATAAGGAATTCGAGCAGGTAAGGACATCAAGC
>KM597059
CCAATGCTATGAGTCTGCTGAGCCACCAGGCCCTTGTGTGTAGGTTACCTACACACACACACACACACACA
CACACACACACACACACACACACACACAAAAACATGGAGCTGACATTTGGATTATCAGATGTCACTTCTAG
AATAAATGAAATCACAGCATCTGAGACGTATCCTGAGAGTAGATAAAAAGACTCCAGCTTTTGCCCTACGG
AATGAA
>KM597060
ACCACCAGTCATCTCTCTTTCCTACACTCAGGGCAGCTCACTCTCTAATACAAAAATAAAATAAAATTCAAA
GCAGTGCAAAACACACACACACACACACATGCTATGTGCTATATATGCTATGTAGCATATATAGCATGCATCT
GTCTGTCTGTCTGTCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTAACTA
GACAACTTGAATCTCCAGCATA
>KM597061
CTGGATGTGGTTCTCAGTGGGCCCTTGGCTGAACATAGTAAGAATTGCACACATGTGACAATTTTGAGATAT
GGAAATATAGATAGATAGATGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGA
TAGATAGACAGACAGATTGATCTAGGTCTTCAAATATAGACAATCTTTATAATTCGTGGGTTCTATATTTGCA
AATTTGCCTACACGCTACAATTTCTTT
>KM597062
TGAATTGGTAGAGTTTGGTGTGCCGGTACCGACATTTGGAGTTTGCAGGACTGCTTGCTTCTTCTTTATTTG
TGGACTCAATACCTTGTTCCTGACTTTGTGGACTCATTGTTTGTTCCTTGGTTCATGGACACATTTCCCTGTT
CCTTTGTTTTGCTGGACTGGGCGCAGCCAGAGGCTGCTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG
TGTGTGTGTGTGCCTTGCTCTGGGACTTGC
>KM597063
GGCTTGGCTTGACATGCTAGGTCTTATTTTTGGGATAGGTTTTATTTTCAGGGAAACATCAGATAGATAGATA
GAAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGAT
AGATAGATAGATATGGTTCAAGACCACACAGGCATTCCAAAACACAACCTACTCATTGTTGGAAGAGAAGA
AAGTCTAGGCCCAGAGACCCCCCAGAGATACGGAGGATTCCAGA
>KM597064
CCATCTAGCAGCAGAGTTCACCACATGGCACCTGGGGATATTACCTCACTCAGCACAGTTCAATATGACTTA
TCTCAGACAAACTCCTAGGATTTGCACATGAGCCCTACAAAACAACCAATAGGATACATTTATTGCACAGGA
ATAGGAAGCTCTAGGGCAAGGCCCAAGAGGGTATATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTA
TCTATCTATCTATCTATCTATCTATCATCTCCACTCCACTCCACTCCACTC